



## City Research Online

### City, University of London Institutional Repository

---

**Citation:** Davenport, J. J., Hickey, M., Phillips, J. P. & Kyriacou, P. A. (2017). Dual pO<sub>2</sub>/pCO<sub>2</sub> fibre optic sensing film. *The Analyst*, 142(10), pp. 1711-1719. doi: 10.1039/C7AN00173H

This is the accepted version of the paper.

This version of the publication may differ from the final published version.

---

**Permanent repository link:** <https://openaccess.city.ac.uk/id/eprint/17560/>

**Link to published version:** <https://doi.org/10.1039/C7AN00173H>

**Copyright:** City Research Online aims to make research outputs of City, University of London available to a wider audience. Copyright and Moral Rights remain with the author(s) and/or copyright holders. URLs from City Research Online may be freely distributed and linked to.

**Reuse:** Copies of full items can be used for personal research or study, educational, or not-for-profit purposes without prior permission or charge. Provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

---

---



# Dual $p\text{O}_2/p\text{CO}_2$ fibre optic sensing film

John J. Davenport, \* Michelle Hickey, Justin P. Phillips and Panayiotis A. Kyriacou

A fibre optic multi-sensor has been developed for biomedical sensing applications using a tip coating solution sensitive to both oxygen and carbon dioxide. An oxygen sensitive phosphorescence quenching complex based on platinum octaethylporphyrin (PtOEP) was combined with a carbon dioxide sensitive phosphorescence compound based on 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS). When excited by blue light (470 nm), the resultant coating had two fluorescent peaks at 515 nm (green) and 645 nm (red) which responded to partial pressure of  $\text{CO}_2$  and  $\text{O}_2$  respectively. The sensor was tested *in vitro* and shown to be able to measure  $\text{CO}_2$  and  $\text{O}_2$  simultaneously and in real time, with calibration constants of  $0.0384 \text{ kPa}^{-1}$  and  $0.309 \text{ kPa}^{-1}$  respectively. The  $\text{O}_2$  sensitive peak received some overlap from the 515 nm peak (0.38% of peak intensity) as well as some cross-sensitivity (maximum, 5.1 kPa  $p\text{CO}_2$  gave a measurement equivalent to 0.43 kPa of  $\text{O}_2$ , a ratio of 0.08 : 1). However, these effects can be subtracted from measurements and no significant cross-sensitivity or overlap was seen in  $\text{CO}_2$  measurements from  $\text{O}_2$ . This novel compound presents great potential for use in medical sensors and we expect it to be important to a wide range of future applications.

## 1. Introduction

### Background

In this study a fibre-optic sensor has been developed using a novel chemical coating sensitive to both  $p\text{O}_2$  and  $p\text{CO}_2$ , allowing simultaneous measurements with a single system. Previous studies have demonstrated fibre optic sensors for measurement of  $p\text{O}_2$ <sup>1–3</sup> and  $p\text{CO}_2$ <sup>4–6</sup> in isolation, while some sensors and coating layers have been developed into commercially available products.<sup>7–10</sup> Fibre optic sensors have advantages over other types of sensors in that they are chemically inert, able to reach difficult locations, and provide electrical isolation from optoelectronic components, reducing the risk of electrocution. These advantages are particularly useful for biomedical applications.

Despite clear potential benefits,  $p\text{O}_2$  and  $p\text{CO}_2$  sensors have not been combined onto a single optical fibre. In this study we have developed a novel sensor which uses a chemical coating containing both  $p\text{O}_2$  and  $p\text{CO}_2$  sensitive materials. Combining measurements of both gases in a single fibre allows more comprehensive assessment of respiratory gas exchange in biological tissue. Simultaneous measurement of  $p\text{O}_2$  and  $p\text{CO}_2$  will provide information regarding the uptake of oxygen and carbon dioxide production within biological systems, which will provide insight into respiratory and metabolic processes. The combination of

both measurements suggest multiple laboratory applications including measurements of cellular respiration *in vitro*, as well as analysis of gas partial pressures in blood and body fluid samples. It will also find multiple applications as an *in vivo* biomedical sensor, particularly monitoring  $p\text{O}_2$  and  $p\text{CO}_2$  in the interstitial fluid. The sensor described in this paper was developed with this application in mind, specifically to provide continuous monitoring of  $p\text{O}_2$  and  $p\text{CO}_2$  in the gastrointestinal epithelium for estimation of the perfusion status of the gut. Such measurements provide early warning of impending gut ischaemia which can lead to deterioration of intensive care patients' conditions including development of sepsis, septic shock and multiple organ dysfunction.<sup>11</sup> Other applications could include transcutaneous gas concentration measurements,<sup>12,13</sup> as well as measurement of inspired and expired respiratory gas concentrations,<sup>14</sup> either incorporated into an anaesthetic breathing circuit or directly from the mouth, nose or trachea.

Chen *et al.*<sup>15</sup> developed a fibre optic  $p\text{O}_2$  sensor based on PtOEP and a cylindrical core optical fibre. The sensor was optimised for rapid measurements showing a minimum response time of less than 50 ms. Yang *et al.*<sup>16</sup> developed a  $p\text{O}_2$  sensor on microstructured polymer optical fibre segments tris (4,7-diphenyl-1,10-phenanthroline) ruthenium(II) dichloride ( $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$ ). The sensor gave a ratio of fluorescent intensities between pure  $\text{N}_2$  and pure  $\text{O}_2$  of 10.8. Bukowski *et al.*<sup>17</sup> developed a sensor material based on tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) ( $[\text{Ru}(\text{dpp})_3]^{2+}$ ). Coated onto a microscope slide it gave a ratio of fluorescent intensities between pure  $\text{N}_2$  and pure  $\text{O}_2$  of 35.

For  $p\text{CO}_2$ , Chu *et al.*<sup>4</sup> Developed a fibre optic sensor based on 1-hydroxy-3,6,8-pyrenetrisulfonic acid trisodium salt (HPTS), finding a limit of detection of 0.03%. Contreras-Gutierrez *et al.*<sup>18</sup> developed a  $p\text{CO}_2$  fibre optic sensor using a polymer matrix in which a fluorescent dye is combined directly with the polymer molecule, showing a limit of detection of 0.4%  $\text{CO}_2$ . Segawa *et al.*<sup>19</sup> developed a similar fibre optic sensor using thymol blue with a minimum detected  $\text{CO}_2$  concentration of 0.2%.

This study builds on the work of Chen *et al.* and Chu *et al.* develop a novel chemical mix which, when coated onto the tip of an optical fibre, produces a sensor capable of responding to both  $p\text{O}_2$  and  $p\text{CO}_2$  simultaneously. The coating avoids adverse interactions between the various components involved and maintains performance with minimal cross-sensitivity. This development presents many new opportunities for biomedical and other sensing applications.

## 2. Theory

### $p\text{O}_2$ measurement

Measurement of the partial pressure of oxygen in this system is based on phosphorescence quenching, using the molecule PtOEP. Phosphorescence occurs when a molecule, referred to as a fluorophore, absorbs a photon of one wavelength, entering an excited state, and then re-emits a photon of a different wavelength at a later point. The absorption and the re-emission wavelengths are both characteristic of the specific molecule.

Phosphorescence quenching occurs when excitation energy is transferred from the fluorophore to another chemical, referred to as a quencher. The fluorophores are therefore unable to emit phosphorescent photons and the intensity of phosphorescent light decreased.

There are many mechanisms for this energy transfer including collision quenching, charge transfer or the formation of static complexes. Here, we focus on collision quenching, one of the most common types and the mechanism responsible for oxygen quenching of PtOEP.<sup>15</sup> In collision quenching the fluorophore and the quencher interact and energy is transferred to the quencher. This prevents the fluorophore decaying photo-chemically and emitting its photons.

By observing the decrease in phosphorescence intensity, the concentration of the quencher can be ascertained. In the simplest case of a single collision-quenching path in a homogeneous environment, the phosphorescence intensity change caused by quenching is given by:<sup>15</sup>

$$I = \frac{I_0}{1 + K_{sv}[\text{O}_2]} \quad (1)$$

where  $I$  is the phosphorescence intensity in the presence of oxygen,  $I_0$  is the intensity in its absence,  $K_{sv}$  is the Stern-Volmer constant and  $[\text{O}_2]$  is the oxygen concentration.  $K_{sv}$  is specific to the combination of fluorophore and quencher and

is equal to the product of  $\tau_0$ , the lifetime of the excited fluorophore and  $k$ , the bi-molecular rate.

The values of  $I_0$  and  $K_{sv}$  depend on the intensity and coupling efficiency of the excitation light source, the quantity and concentration of the fluorophore, temperature of the environment and the specific efficiency of collisions between PtOEP and oxygen. The values of  $I_0$  and  $K_{sv}$  can be found for a given device to allow oxygen concentration to be calculated.

### $p\text{CO}_2$ measurement

Based on phosphorescence indication of pH changes associated with dissolved  $\text{CO}_2$ . The fluorophore can absorb light of 480 nm and re-emit light of 520 nm.<sup>18</sup> In the presence of  $\text{CO}_2$  and a transfer agent, a series of equilibrium reactions take place, decreasing the concentration of the fluorophore. The concentration of  $\text{CO}_2$  can be found from the intensity of the emitted light.

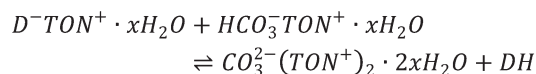
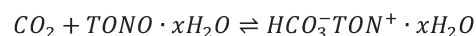
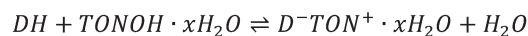
The interaction between the fluorophore, TONOH and dissolved  $\text{CO}_2$  is shown in Scheme 1.<sup>20</sup> Here, DH is the protonated form of the fluorophore and  $\text{D}^-$  is the deprotonated form.

The first line shows the interaction between the TONOH and the fluorophore before the sensor is exposed to the sample. The second line shows the interaction between  $\text{CO}_2$  and TONOH. The third line shows the interaction between the reaction products of the first two lines. Therefore, when TONOH is abundant, the ratio of DH to  $\text{D}^-$  is dependent on the quantity of  $\text{CO}_2$ .<sup>20</sup>

When excited, DH fluoresces at 520 nm. The greater the concentration of excited fluorescent molecules, the greater the phosphorescence intensity. Provided sufficient TONOH was included, by shifting the concentration of DH,  $\text{CO}_2$  decreases the intensity of fluorescent light resulting from excitation. The resultant intensity follows a relationship similar to the Stern-Volmer relationship described above.<sup>4</sup>

$$I = \frac{I_0}{1 + \alpha[\text{CO}_2]} \quad (2)$$

where  $[\text{CO}_2]$  is the carbon dioxide concentration,  $\alpha$  is a calibration constant incorporating equilibrium constants for the interactions shown in Scheme 1, and other symbols have the same meanings as above. The values of  $I_0$  and  $\alpha$  can be found for a given device to allow carbon dioxide concentration to be calculated.



**Scheme 1** Chemical interaction between the fluorophore, TONOH and dissolved  $\text{CO}_2$ .

### 3. Materials and methods

#### Sensor fabrication and setup

The combined  $p\text{CO}_2/p\text{O}_2$  sensor was made by dip coating a polymer layer containing materials sensitive to  $\text{CO}_2$  and to  $\text{O}_2$  onto the distal tip of an optical fibre. The tip was then placed in contact with a sample. The interaction between the sensing layer and  $\text{O}_2$  or  $\text{CO}_2$  in the sample allowed the partial pressures of the two gases to be measured.

Fig. 1 shows a diagram of the distal tip of the sensor. The sensing layer contains PtOEP, a fluorescent molecules sensitive to  $p\text{CO}_2$  and HPTS, a fluorescent molecule that can be transferred to a non-fluorescing form by  $\text{CO}_2$  when in the presence of the transfer agent TONOH. Both molecules were excited by blue (460 nm) light, provided by an LED coupled to the proximal end of the fibre. Excited PtOEP fluoresced red (645 nm) depending on the concentration of  $\text{O}_2$  and HPTS fluoresced green (515 nm) depending on the concentration of  $\text{CO}_2$ . The fluorescent wavelength of 515 nm observed in this study is slightly shorter than usual HPTS peak wavelength of 520 nm. This difference is discussed in more detail later. By analysing the spectrum of the light returning from the sensor, the partial pressure of  $\text{CO}_2$  and  $\text{O}_2$  could be ascertained.

#### Preparation and application of the sensing film

The following chemicals were used to create the coating solution: platinum octaethylporphyrin (PtOEP,  $\text{Pt}(\text{NC}_9\text{H}_{11})_4$ , CAS: 31248-39-2) as the oxygen sensitive fluorophore, poly(ethyl methacrylate) (PEMA,  $[\text{CH}_2\text{C}(\text{CH}_3)(\text{CO}_2\text{C}_2\text{H}_5)]_n$ , CAS: 9003-42-3) as a fixer, dichloromethane ( $\text{CH}_2\text{Cl}_2$  CAS: 75-09-2) as a solvent, 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS,  $\text{C}_{16}\text{H}_7\text{Na}_3\text{O}_{10}\text{S}_3$ , CAS: 6358-69-6) as the  $\text{CO}_2$  sensitive fluorophore, 1-ethyl-3-methylimidazolium tetrafluoroborate (EMIMBF<sub>4</sub>, CAS: 143314-16-3) to stabilise the HPTS and tetraoctylammonium hydroxide solution (TONOH,  $\text{C}_{32}\text{H}_{69}\text{NO}$ , CAS: 17756-58-0, supplied in 20% solution with methanol) as a phase transfer agent. All three were obtained from Sigma-Aldrich Corporation, St Louis, USA.

PtOEP (1.0 mg) and PEMA (102 mg) powder was mixed with  $\text{CH}_2\text{Cl}_2$  liquid (1.6 ml) in a glass vial, which was left to stand

until the powders had completely dissolved. HPTS (5.0 mg) powder was then added. The ionic liquid EMIMBF<sub>4</sub> (0.1 ml) was added to stabilise the HPTS and increase its lifetime, as described by Oter *et al.*<sup>21</sup> Finally, TONOH solution (0.4 ml) was added and the vial was left to stand to allow the HPTS to dissolve.

The chemical coating was applied using a precision dip coater (precision dip coater, model – QPI-168, Qualtech Products Industry, Denver, USA). It was set to lower the tip of the optical fibre (BFL48-600 fibre, 600  $\mu\text{m}$  fibre core, 0.48 NA, Ocean Optics, Dunedin, USA) into a vial of coating solution and then withdraw it at a rate of 1  $\text{nm s}^{-1}$ , allowing 30 minutes for the solvent to evaporate. After which, a coating of coat of silicone rubber was applied (CS2 condensation cure, Easy Composites, Stoke-on-Trent, UK, using MED-160 primer, Nusil Silicon Technology, Carpinteria, USA) and left to set for 24 hours.

#### Chemical mix development

The chemical mix used to create the sensing was described above. Before this mix was finalised a number of other preliminary alternatives were tried in order to ensure the composition worked and performed as desired. Table 1 shows the makeup of the mixes that were tested before finalising the mix used for this study.

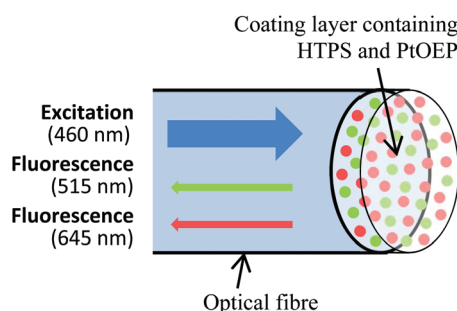
Early iterations of the mix, the  $\text{CO}_2$  signal at 515 nm decayed rapidly, within a few hours of use, limiting the function of the sensor. Varying the concentration of EMIMBF<sub>4</sub> helped to increase the lifetime of the sensor, but had the potential of forcing the PEMA out of solution where it formed solid crystals in mixing vial.

The order at which components were added was also important. It was found that PtOEP would not dissolve in a solution containing methanol, such as that added with the TONOH, but it would remain in solution if first dissolved in  $\text{CH}_2\text{Cl}_2$  in advance of adding methanol (at least in the concentrations used here).

Finally, once a working mix had been obtained, several more compositions were tested in order to optimise the performance and maximise the strength of  $\text{CO}_2$  and  $\text{O}_2$  signals. By increasing the intensity of return signals from the sensor, its accuracy and limit of detection was expected to increase.

Numerous studies have been done on theoretically or experimentally determining the thickness of a smooth film dip-coated onto a surface,<sup>22,23</sup> however they typically assume a vertical surface withdrawn from the coating. Fig. 2 shows a microscope image of the chemical mix coated onto the tip of an optical fibre. It can clearly be seen that surface is not flat or smooth with numerous creases.

It is believed that these are caused by a small droplet forming at the tip of the fibre, which then shrinks as the solvents evaporate. Thakur *et al.*<sup>24</sup> tested a PEMA based coating similar to the one described in this study, deposited by spray pyrolysis. Similar creases were observed when zing oxide nanoparticles were introduced, also attributed to shrinkage during evaporation of the solvent. Thakur *et al.* observed a refractive



**Fig. 1** The distal tip of the sensor, including a coating layer containing HPTS and PtOEP molecules. Both are excited by blue (460 nm) light. HPTS fluoresces green (515 nm), decreasing with increasing  $p\text{CO}_2$ . PtOEP fluoresces red (645 nm) decreasing with increasing  $p\text{O}_2$ .

**Table 1** Details of preliminary chemical mix compositions tested before finalising to the mix used in this study. Components were added in the quantities and orders listed, and solids were given time to dissolve between each step they were added to a solvent

Batch	Details	Observations
1a	0.7 mg PtOEP, 1.6 ml CH <sub>2</sub> Cl <sub>2</sub> , 0.1 ml EMIMBF <sub>4</sub> , 102 mg PEMA, 0.4 ml TONOH in methanol, 40 mg HPTS	Strong CO <sub>2</sub> peak that decayed rapidly (few hours).
1b	0.7 mg PtOEP, 100 mg PEMA, 9.8 mg HPTS, 1.6 ml CH <sub>2</sub> Cl <sub>2</sub> .	Solids did not dissolve fully.
1c	0.5 mg PtOEP, 102 mg PEMA, 1.6 ml CH <sub>2</sub> Cl <sub>2</sub> , 0.1 ml EMIMBF <sub>4</sub> , 5 mg HPTS.	—
1d	0.1 mg PtOEP, 101 mg PEMA, 1.6 ml CH <sub>2</sub> Cl <sub>2</sub> , 0.1 ml EMIMBF <sub>4</sub> , 1.5 mg HPTS.	HPTS did not dissolve.
1e	0.1 mg PtOEP, 100 mg PEMA, 1.6 ml CH <sub>2</sub> Cl <sub>2</sub> , 1.5 mg HPTS, 0.4 ml TONOH in methanol, 0.1 ml EMIMBF <sub>4</sub> .	HPTS appeared to dissolve better after TONOH was added.
1f	0.9 mg PtOEP, 101 mg PEMA, 1.6 ml CH <sub>2</sub> Cl <sub>2</sub> , 0.4 ml TONOH in methanol, 105 mg Polym H7, a matrix forming polymer bonded to fluorescent molecules as well as TONOH.	CO <sub>2</sub> peak decays. A further 0.1 ml EMIMBF <sub>4</sub> , peak still decays. CO <sub>2</sub> peak decayed.
1g	0.9 mg PtOEP, 100 mg PEMA, 0.4 ml CH <sub>2</sub> Cl <sub>2</sub> , 1.2 ml methanol, 0.4 ml TONOH in methanol, 1.5 mg HPTS.	PEMA crystals formed on adding methanol, but dissolved on shaking. No O <sub>2</sub> peak, CO <sub>2</sub> peak decayed
1gII	0.6 mg PtOEP, 100 mg PEMA, 0.4 ml CH <sub>2</sub> Cl <sub>2</sub> , 1.2 methanol, 0.1 ml EMIMBF <sub>4</sub> , 2 mg HPTS.	As before, PEMA crystals formed but dissolved when shaken.
2h	—	—
2a	1 mg PtOEP, 0.2 ml EMIMBF <sub>4</sub> , 1.5 ml CH <sub>2</sub> Cl <sub>2</sub> , 0.2 ml TONOH in methanol.	—
2b	Vial 1: 102 mg PEMA, 1 mg PtOEP, 0.4 ml CH <sub>2</sub> Cl <sub>2</sub> . Vial 2: 0.2 ml EMIMBF <sub>4</sub> , 1.5 ml methanol, 0.2 ml TONOH in methanol. Vial 1 then added to vial 2, then 1.7 mg HPTS	—
2c	Vial 1: 100 mg PEMA, 1 mg PtOEP, 0.4 ml CH <sub>2</sub> Cl <sub>2</sub> . Vial 2: 1.5 ml methanol, 0.2 ml TONOH in methanol.	—
2d	100 mg PEMA, 0.5 mg PtOEP, 0.7 mg HPTS, 0.4 ml CH <sub>2</sub> Cl <sub>2</sub> , 1.2 methanol, 0.4 ml TONOH in methanol, 0.1 ml EMIMBF <sub>4</sub> .	HPTS did not dissolve. Some of the PEMA solidified or did not dissolve initially.
2e	101 mg PEMA, 0.5 mg PtOEP, 0.4 ml CH <sub>2</sub> Cl <sub>2</sub> , 0.1 ml EMIMBF <sub>4</sub> , 1.5 ml methanol, 0.2 ml TONOH in methanol, 0.1 mg HPTS.	Gave low but stable CO <sub>2</sub> peak, no O <sub>2</sub> signal.
2f	103 mg PEMA, 1.4 mg PtOEP, 0.4 ml CH <sub>2</sub> Cl <sub>2</sub> , 1.5 ml methanol, 0.2 ml EMIMBF <sub>4</sub> , 0.2 ml TONOH in methanol, 0.1 mg HPTS.	Low CO <sub>2</sub> signal, small O <sub>2</sub> peak.
2g	100 mg PEMA, 2 mg PtOEP, 0.5 ml CH <sub>2</sub> Cl <sub>2</sub> , 1.5 ml methanol, 0.2 ml EMIMBF <sub>4</sub> , 0.2 ml TONOH in methanol, 0.1 mg HPTS.	No signal at all.
2h	100 mg PEMA, 0.1 mg PtOEP, 0.5 ml CH <sub>2</sub> Cl <sub>2</sub> , 1.5 ml methanol, 0.2 ml EMIMBF <sub>4</sub> , 0.2 ml TONOH in methanol, 0.2 mg HPTS.	Medium signal for both CO <sub>2</sub> and O <sub>2</sub> .
3	1000 mg PEMA, 1.5 mg PtOEP, 5 ml CH <sub>2</sub> Cl <sub>2</sub> , 15 ml methanol, 2 ml EMIMBF <sub>4</sub> , 2 ml TONOH in methanol, 8 mg HPTS.	Similar behaviour to 5p.
4	9.9 mg PtOEP, 1003 mg PEMA, 16 ml CH <sub>2</sub> Cl <sub>2</sub> , 15 mg HPTS, 1 ml EMIMBF <sub>4</sub> , 4 ml TONOH in methanol.	—
4a	3 mg PtOEP, 101 mg PEMA, 1.6 ml CH <sub>2</sub> Cl <sub>2</sub> , 15 mg HPTS, 0.1 ml EMIMBF <sub>4</sub> , 0.4 ml TONOH in methanol.	—
4b	1 mg PtOEP, 102 mg PEMA, 1.6 ml CH <sub>2</sub> Cl <sub>2</sub> , 5 mg HPTS, 0.1 ml EMIMBF <sub>4</sub> , 0.4 ml TONOH in methanol.	Gives strong and stable peaks for both CO <sub>2</sub> and O <sub>2</sub> .
4c	1 mg PtOEP, 100 mg PEMA, 0.4 ml CH <sub>2</sub> Cl <sub>2</sub> , 5.1 mg HPTS, 0.1 ml EMIMBF <sub>4</sub> , 0.4 ml TONOH in methanol, 0.2 methanol	No O <sub>2</sub> peak.
4d	1 mg PtOEP, 100 mg PEMA, 1.6 ml CH <sub>2</sub> Cl <sub>2</sub> , 5 mg HPTS, 0.2 ml EMIMBF <sub>4</sub> , 0.4 ml TONOH in methanol	—



**Fig. 2** Microscope image (20x magnification) of the chemical mix coated onto the tip of a 600 μm optical fibre.

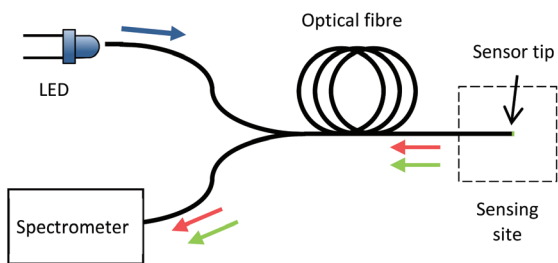
index of PEMA coatings to vary between 1.55 and 1.59, depending on the deposition time of the coating.

These may influence the performance of the sensors as light reaching the surface will tend to scatter rather than reflect evenly. However, this sensor method does not rely on reflection or coating thickness for its function, rather that excitation light reaches the HPTS and PtOEP, and phosphorescent light transmits, reflects or scatters back. Variations in the surface properties of the coating are expected to affect only the calibration of the sensor, not its basic function.

#### System setup

Sensors were mechanically spliced to an optical fibre splitter (bifurcated borosilicate fibre, 600 μm diameter multi-mode





**Fig. 3** Diagram of the optical layout of the sensor. The fibre splitter allows light from the LED to reach the sensing layer and excite the fluorophores, and fluorescent light to pass back to the spectrometer.

core, BIFBORO-600-2 supplied by Ocean Optics) connected to an LED (460 nm, Wurth Elektronik, 151033BS03000, RS Components, UK) and a spectrometer (Ocean Optics Flame miniature spectrometer), using a 50 ms integration time and averaging over 100 measurements throughout this study. Fig. 3 shows a diagram of the optical layout of the system.

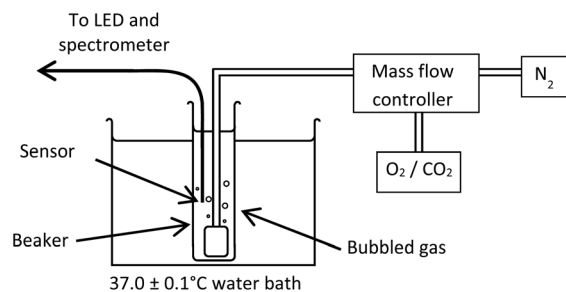
Blue light (460 nm) was introduced to the optical fibre using an LED, which then passed through the fibre splitter to the sensing layer. The LED had a luminous intensity of 3.8 cd and couples an estimated 1.4 mW of light into the optical fibre. The fluorescent components of HPTS fluoresced green (515 nm) with decreasing intensity in the presence of  $\text{CO}_2$ . PtOEP fluoresced red (645 nm) with decreasing intensity in the presence of  $\text{O}_2$ . The fibre splitter delivered this light to the spectrometer. By measuring the intensity of green and red light from the sensor the partial pressure of  $\text{CO}_2$  and  $\text{O}_2$  to be ascertained.

### *In vitro* testing

The sensors were tested *in vitro*. The sensor was inserted into a narrow beaker containing distilled water in order to measure the effects of changing  $p\text{O}_2$  and  $p\text{CO}_2$ . The partial pressures were controlled by bubbling gas through an aerator and allowing the dissolved gases to equilibrium. The gas input was provided by combining flow from an  $\text{N}_2$  cylinder, a  $\text{CO}_2$  cylinder and an  $\text{O}_2$  cylinder (supplied by BOC, USA). The beaker was placed within a water bath (Fisher Scientific 12777819 – Thermostatic circulator bath) kept at  $37.0 \pm 0.1^\circ\text{C}$ . This mimicked human body temperature as well as ensuring that temperature changes did not affect the sensing layer. A diagram of the *in vitro* setup is shown in Fig. 4.

Flow rates were measured and controlled using mass flow controllers (FMA-A2406-SS-( $\text{N}_2$ ) mass flow controller, supplied by Omega Engineering, US). Concentrations of  $\text{CO}_2$  and  $\text{O}_2$  were controlled by varying the ratios of flow with  $\text{N}_2$ . Preliminary testing found that with this setup, dissolved gas concentrations reached equilibrium within three minutes. Five minutes was therefore given to allow a margin of error. A baseline measurement of 100%  $\text{N}_2$  was recorded between each  $\text{O}_2$  or  $\text{CO}_2$  measurement.

First, a sensor was tested using under varying oxygen conditions whilst keeping the carbon dioxide concentration at



**Fig. 4** Diagram of the setup used for in-vitro testing of the sensors. Oxygen concentration was controlled by bubbling gas through the water in the conical flask.

zero, followed by testing a sensor under varying carbon dioxide conditions while keeping the oxygen concentration at zero. This allowed the sensitivity to oxygen and carbon dioxide to be tested separately, and cross-sensitivity of each of the fluorescent peaks to be identified.

Details of the flow rates used for these tests are given in Tables 2 and 3. Partial pressures were calculated from gas percentages assuming a standard atmospheric pressure of 101.3 kPa.

## 4. Results

Fig. 5 shows the spectrum of light returning from the sensor at varying levels of  $p\text{CO}_2$ , normalised to the excitation light from

**Table 2** Variation  $p\text{O}_2$  in determined by flow rates of  $\text{O}_2$  and  $\text{N}_2$ . Each gas mix was permitted to flow for five minutes to allow the concentration in the water to reach equilibrium and a baseline measurement of 100%  $\text{N}_2$  was recorded between each  $\text{O}_2$  measurement

$\text{O}_2$ ( $\text{ml min}^{-1}$ )	$\text{N}_2$ ( $\text{ml min}^{-1}$ )	$\text{O}_2$ (%)	$p\text{O}_2$ (kPa)
0	500	$0 \pm 3$	$0 \pm 3$
100	400	$20 \pm 3$	$20 \pm 3$
200	300	$40 \pm 3$	$41 \pm 3$
300	200	$60 \pm 3$	$61 \pm 3$
400	100	$80 \pm 3$	$81 \pm 3$
450	50	$90 \pm 3$	$91 \pm 3$
500	0	$100 \pm 3$	$101 \pm 3$

**Table 3** Variation  $p\text{CO}_2$  in determined by flow rates of  $\text{CO}_2$  and  $\text{N}_2$ . Each gas mix was permitted to flow for five minutes to allow the concentration in the water to reach equilibrium and a baseline measurement of 100%  $\text{N}_2$  was recorded between each  $\text{CO}_2$  measurement

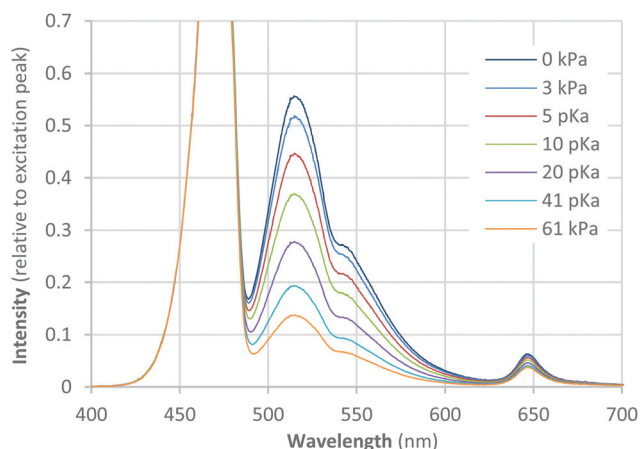
$\text{CO}_2$ ( $\text{ml min}^{-1}$ )	$\text{N}_2$ ( $\text{ml min}^{-1}$ )	$\text{CO}_2$ (%)	$p\text{CO}_2$ (kPa)
0	500	$0 \pm 3$	$0 \pm 3$
15	485	$3 \pm 3$	$3.0 \pm 3$
25	475	$5 \pm 3$	$5.1 \pm 3$
50	450	$10 \pm 3$	$10 \pm 3$
100	400	$20 \pm 3$	$20 \pm 3$
200	300	$40 \pm 3$	$41 \pm 3$
300	200	$60 \pm 3$	$61 \pm 3$

the LED is centred at 470 nm reflected from the sensor tip. The peak from the LED is truncated in this graph as it is not the focus of this study. The phosphorescence signal from the CO<sub>2</sub> sensitive material HTPS is centred at 515 nm, where it can be seen to decrease in intensity with increasing concentration, as expected.

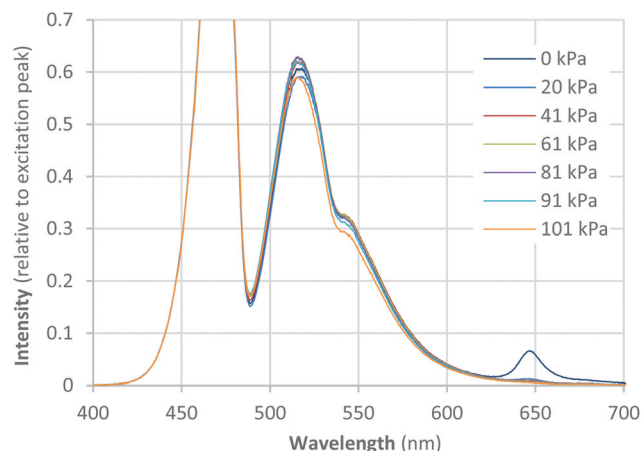
The peak at 645 nm is the phosphorescence signal from the O<sub>2</sub> sensitive material PtOEP. It shows some CO<sub>2</sub> cross-sensitivity to O<sub>2</sub> measurements, which will be discussed in more detail later. It is also noticeable that there is a dip in intensity from the CO<sub>2</sub> sensitive peak centred at around 539 nm which is not usually seen for HTPS.<sup>18</sup> Likewise the HTPS is usually observed at 520 nm as the peak is broader, without the dip at 539 nm.<sup>18</sup> This dip is believed to be caused by absorbance from the PtOEP included in the coating, and it was found to be absent when PtOEP was not included. However, provided measurements were taken from the shifted HTPS peak maximum at 539 nm, it was not found to be an issue.

The sensor spectrum for varying concentrations of oxygen is shown in Fig. 6. It can be seen that the 515 nm peak does not decrease as seen in Fig. 5, showing minimal O<sub>2</sub> cross-sensitivity to CO<sub>2</sub> measurements. The 645 nm is shown in more detail in Fig. 7 where it can be seen to drop off rapidly with increasing O<sub>2</sub> as expected. It can also be seen that there is a slight overlap between the 645 nm peak and the tail end of the 539 nm peak, which may lead to further cross-sensitivity.

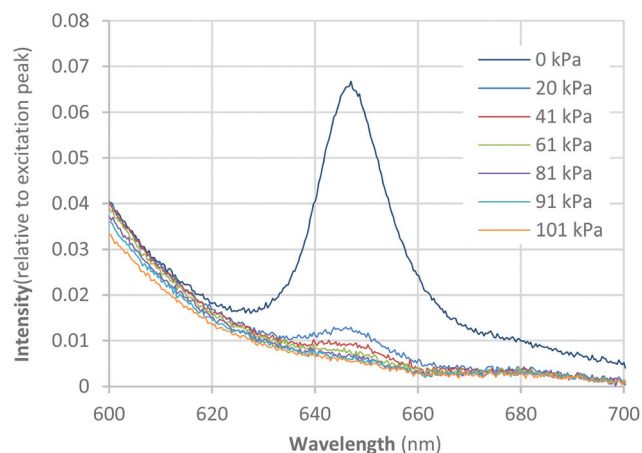
Fig. 8 shows the intensity ration plot for the 515 nm peak for varying levels of  $p\text{CO}_2$ , along with a theoretical plot based on eqn (2). The results correspond well to theoretical predictions, with all values being within errors. Errors were found by combining the standard deviation of all baseline peak intensities with the standard deviation of phosphorescence intensity change measurements over a 3.1 nm range at the top of each peak. The calibration constant,  $\alpha$ , was found empirically by a least squares fit to have a value of  $0.0384 \text{ kPa}^{-1}$ .



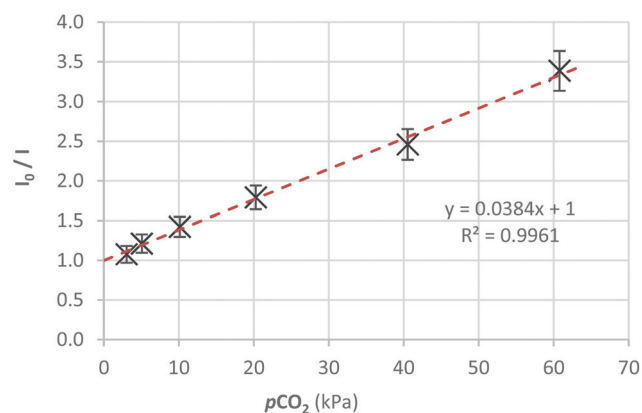
**Fig. 5** Spectrum of light returning from the sensor at varying  $p\text{CO}_2$ . The truncated peak at 470 nm is the excitation peak from the LED reflecting off the sensor tip, the peak at 515 nm is the phosphorescence peak sensitive to CO<sub>2</sub> and the peak at 645 nm is the phosphorescence peak sensitive to O<sub>2</sub>.



**Fig. 6** Spectrum of light returning from the sensor at varying  $p\text{O}_2$ . The truncated peak at 470 nm is the excitation peak from the LED reflecting off the sensor tip, the peak at 515 nm is the phosphorescence peak sensitive to CO<sub>2</sub> and the peak at 645 nm is the phosphorescence peak sensitive to O<sub>2</sub>.

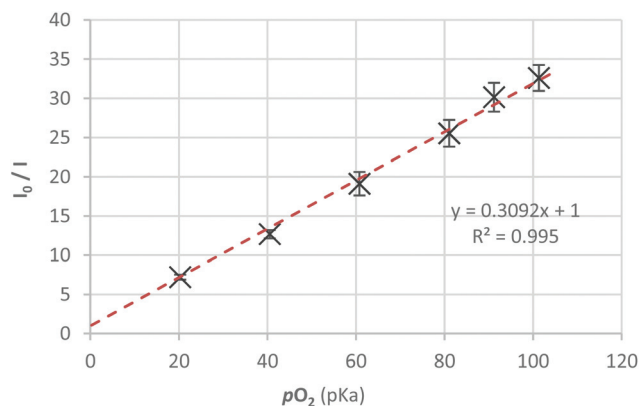


**Fig. 7** Expansion of the O<sub>2</sub> sensitive phosphorescence peak at 645 nm at varying  $p\text{O}_2$ . The tip of the CO<sub>2</sub> sensitive peak can also be seen on the left side.



**Fig. 8** Plot of the CO<sub>2</sub> sensitive phosphorescence peak at 515 nm against varying  $p\text{CO}_2$ .  $\alpha = 0.0384 \text{ kPa}^{-1}$ .



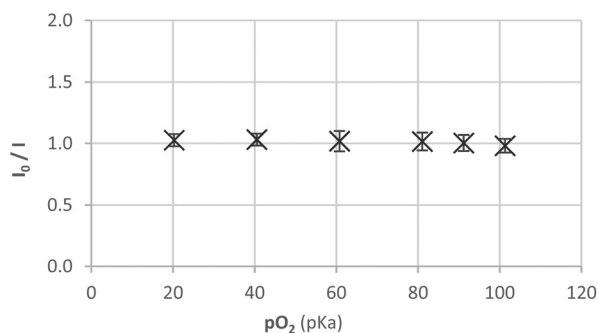


**Fig. 9** Stern-Volmer plot of the  $O_2$  sensitive phosphorescence peak at 645 nm against varying  $pO_2$ .  $K_{sv} = 0.309 \text{ kPa}^{-1}$ . Overlap from the 515 nm peak was subtracted from intensity values before plotting.

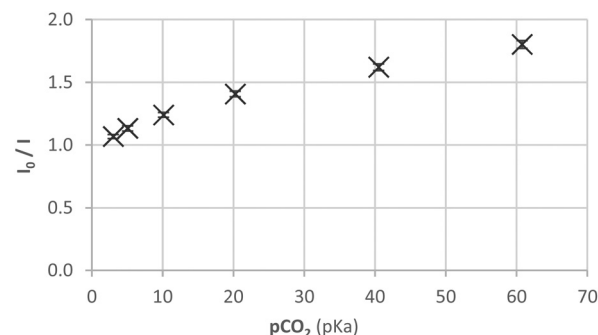
The intensity change for the 645 nm peak for varying concentrations of  $O_2$  is shown in Fig. 9, along with a theoretical Stern-Volmer plot. The overlap from the 515 nm peak shown in Fig. 6 and 7 was removed from 645 nm intensity measurements by subtracting an empirically found factor of  $3.8 \times 10^{-3}$  multiplied by the intensity measured at 515 nm. Figure errors and  $K_{sv}$  were found in the same way as for Fig. 8. Again, measurements correspond well to theoretical predictions, although the small differences in values for high  $O_2$  concentrations are not always distinguishable over errors. The value of  $K_{sv}$  was found to be  $0.309 \text{ kPa}^{-1}$  in this case.

In order to investigate the  $O_2$  cross-sensitivity to  $CO_2$  measurements, the intensity change for the  $CO_2$  sensitive 539 nm peak was measured as for Fig. 5, but with varying concentrations of  $O_2$ . The results are shown in Fig. 10. Intensity changes are very small in comparison to varying  $CO_2$  concentrations and most of them do not show significant change outside of errors. It can be concluded that the  $CO_2$  sensitive peak at 539 nm does not have significant cross-sensitivity to  $O_2$ .

Similarly, the  $CO_2$  cross-sensitivity to  $O_2$  measurements was investigated by measuring the intensity change of the  $O_2$  sensitive peak at 645 nm whilst varying the concentration of  $CO_2$ .



**Fig. 10** Cross-sensitivity of the  $CO_2$  sensitive peak at 515 nm to varying  $pO_2$ .  $I_0/I$  values were calculated in the same way as for Fig. 8.



**Fig. 11** Cross-sensitivity of the  $O_2$  sensitive peak at 645 nm to varying  $pCO_2$ .  $I_0/I$  values were calculated in the same way as for Fig. 9.

The results are shown in Fig. 11. These results do show a cross-sensitivity to  $CO_2$  which, while small in comparison to the sensitivity to  $O_2$ , is still significant. They also deviate from a linear Stern-Volmer plot.

## 5. Discussion and conclusion

The purpose of this study was to produce and test a phosphorescence chemical coating capable of responding separately to partial pressure of oxygen ( $pO_2$ ) and partial pressure of carbon dioxide ( $pCO_2$ ). A novel fibre-optic sensor was produced which included a chemical coating combining the  $pCO_2$  sensitive molecule HPTS with the  $pO_2$  sensitive molecule PtOEP.

The results show that the two fluorescent chemicals can be combined together without a problematic interaction. In early testing the EMIMBF<sub>4</sub> would sometimes form a solid precipitate when added to the mixture, requiring an adjustment of the concentrations. The process described allowed both fluorescent chemicals, the phase transfer and stabilising agents needed for optimal performance, and a coating polymer could all be combined within a single solution.

Further, the peaks of the two fluorescent chemicals can be clearly distinguished from one another and both remain able to fluoresce when mixed with the other. The addition of PtOEP did cause a dip in the HPTS phosphorescence peak at around 539 nm, shifting its intensity maximum from 520 nm to 515 nm. It is possible that this was caused by a non-fluorescent absorption from PtOEP molecules, but the peak could still clearly be distinguished with the new maximum.

The  $CO_2$  sensitive phosphorescence peak was significantly wider than the  $O_2$  sensitive peak at 645 nm, and overlapped it slightly. It was necessary to subtract this overlap from  $O_2$  measurements. However, this did not result in a significant issue as the intensity of the overlap was proportional to the intensity of the 515 nm peak, which was measured through the testing. The 645 nm peak, being narrower and less intense, did not noticeably overlap with the centre of the 515 nm peak.

The two phosphorescence peaks were clearly shown to respond to their respective target,  $pO_2$  and  $pCO_2$ , decreasing in intensity with increasing partial pressure as predicted by the

Stern–Volmer relationship. Both peaks showed good correspondence to predictions in isolation, allowing calibration constants of  $0.0384 \text{ kPa}^{-1}$  and  $0.3092 \text{ kPa}^{-1}$  for  $\text{CO}_2$  and  $\text{O}_2$  respectively.

While  $\text{CO}_2$  measurements showed little or no cross-sensitivity to  $\text{O}_2$ , there was some cross-sensitivity the other way. The highest cross sensitivity was measured at  $5.1 \text{ pK}_a \text{ CO}_2$ , which effected the  $645 \text{ nm}$  measurement equivalent to  $0.43 \text{ pK}_a$  of  $\text{O}_2$ , a ratio of  $0.08 : 1$ . The lowest was measured at  $61 \text{ pK}_a$  which effected the  $645 \text{ nm}$  measurement equivalent to  $23.5 \text{ pK}_a$  of  $\text{O}_2$ , a ratio of  $0.043 : 1$ . However, this cross-sensitivity appeared to follow a predictable pattern across  $p\text{CO}_2$  values, allowing it to be subtracted from  $p\text{O}_2$  measurements.  $p\text{O}_2$  measurements must therefore be corrected for both overlap from the  $515 \text{ nm}$  peak and for cross-sensitivity to  $p\text{CO}_2$ . However, both are based on the  $515 \text{ nm}$  measurement which is independent of  $p\text{O}_2$  and is measured throughout use.

The spectra observed for the  $\text{CO}_2$  and the  $\text{O}_2$  sensitive peaks of the combined mix are similar to those observed in isolation by previous studies, and similarly linear sensitivity plots were recorded.<sup>1,4</sup> However single sensors have typically shown significantly faster response times than those presented here. With an integration time of  $50 \text{ ms}$  and averaging over  $100$  measurements, the combined sensor had a response time of a little over  $5 \text{ s}$ , whereas Chu *et al.*<sup>4</sup> achieved  $p\text{CO}_2$  measurements in  $1.7 \text{ s}$  and Chen *et al.*<sup>15</sup> achieved  $p\text{O}_2$  response times below  $50 \text{ ms}$ . However it is important to note that these studies were aimed at optimising existing techniques of single measurements in isolation, whereas this study is the first to present combined measurements in this manner.

To conclude, this study demonstrates a new chemical compound that combines two fluorescent materials sensitive to  $p\text{O}_2$  and  $p\text{CO}_2$  respectively. The two materials fluoresce in different parts of the visible spectrum, allowing the signals to be distinguished and  $p\text{O}_2$  and  $p\text{CO}_2$  to be measured simultaneously. The  $p\text{CO}_2$  sensitive peak slightly overlaps the  $p\text{CO}_2$  sensitive peak and there is some cross-sensitivity from  $p\text{CO}_2$  to  $p\text{O}_2$ .

Combining the two sensing materials presented many hurdles, depending on the concentrations and chemicals used, solid components would not dissolve, fluorescent intensity would decay too rapidly for practical use, or crystals would form preventing the mix to be used as a coating. However, after careful testing it proved to be possible to produce a mixture which in which both the  $p\text{O}_2$  and the  $p\text{CO}_2$  sensitive reactions could take place, and which could be successfully coated onto a surface such as an optical fibre.

The compound presents new opportunities for use in medical sensors. The key advantage of this sensor is its small size and relative simplicity compared to the two separate sensors that would otherwise be required. As the two fluorescent molecules are excited by the same wavelength, only a single light source is required. Coated onto the tip of an optical fibre, it can allow  $p\text{CO}_2$  and  $p\text{O}_2$  to be monitored simultaneously in remote locations such as the gastrointestinal tract. Alternatively a larger coating area could be used for a

potentially increase sensitivity if accessing a remote location is not required.

The intensity of the fluorescent peaks may be measured using a spectrometer as in this study, or with optical filters and photodiodes. It may also be possible to improve the performance of the  $p\text{O}_2$  measurements by using fluorescent lifetime measurements, which will be the focus of future investigations. This novel compound has great potential which we expect to be important to a wide range of future applications.

## Acknowledgements

This report is independent research funded by the National Institute for Health Research (Invention for Innovation (i4i) program): *Development of a Multi-Parameter Oesophageal Sensor for the Early Detection of Multiple Organ Dysfunction Syndrome (MODS)*, Ref: II-LA-0313-20006. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health.

## References

1. Jiang, L. Gao, W. Zhong, S. Meng, B. Yong, Y. Song, X. Wang and C. Bai, Development of fiber optic fluorescence oxygen sensor in both in vitro and in vivo systems, *Respir. Physiol. Neurobiol.*, 2008, **161**, 160–166.
2. X. Yang, L. Peng, L. Yuan, P. Teng, F. Tian, L. Li and S. Luo, Oxygen gas optrode based on microstructured polymer optical fiber segment, *Opt. Commun.*, 2011, **284**, 3462–3466.
3. J. J. D. M. Hickey, J. P. Phillips and P. A. Kyriacou, Fiber-optic fluorescence-quenching oxygen partial pressure sensor using platinum octaethylporphyrin, *Appl. Opt.*, 2016, **55**(21), 5603–5609.
4. C.-S. Chu and Y.-L. Lo, Fiber-optic carbon dioxide sensor based on fluorinated xerogels doped with HPTS, *Sens. Actuators, B*, 2008, **129**(1), 120–125.
5. P. K. Contreras-Gutierrez, S. Medina-Rodríguez, A. L. Medina-Castillo, J. F. Fernandez-Sanchez and A. Fernandez-Gutierrez, A new highly sensitive and versatile optical sensing film for controlling  $\text{CO}_2$  in gaseous and aqueous media, *Sens. Actuators, B*, 2013, **184**, 281–287.
6. H. Segawa, E. Ohnishi, Y. Arai and K. Yoshida, Sensitivity of fiber-optic carbon dioxide sensors utilizing indicator dye, *Sens. Actuators, B*, 2003, **94**, 276–281.
7. T. Jarm, G. Sersa and D. Miklavcic, Oxygenation and blood flow in tumors treated with hydralazine: Evaluation with a novel luminescence-based fiber-optic sensor, *Technol. Health Care*, 2002, **10**, 363–380.
8. M. Urano, Y. Chen, J. Humm, J. A. Koutcher, P. Zanzonico and C. Ling, Measurements of Tumor Tissue Oxygen Tension Using a Time-Resolved Luminescence-Based Optical OxyLite Probe: Comparison with a Paired Survival Assay, *Radiat. Res.*, 2002, **158**, 167–173.

- 9 Oxford Optronix Ltd., *Oxford Optronix, Sensors, Sensors for Oxygen Monitors*, [Online]. Available: <http://www.oxford-optronix.com/sensor12/sensor-for-Oxygen-Monitors.html>. [Accessed 18th March 2016].
- 10 F. Formenti, R. Chen, H. McPeak, M. Matejovic, A. D. Farmery and C. E. Hahn, A fibre optic oxygen sensor that detects rapid PO<sub>2</sub> changes under simulated conditions of cyclical atelectasis in vitro, *Respir. Physiol. Neurobiol.*, 2014, **191**, 1–8.
- 11 X. Zhang, W. Xuan, P. Yin, L. Wang, X. Wu and Q. Wu, Gastric tonometry guided therapy in critical care patients: a systematic review and meta-analysis, *Crit. Care*, 2015, **19**, 22.
- 12 V. B. Sivarajan and A. D. Bohn, Monitoring of standard hemodynamic parameters: Heart rate, systemic blood pressure, atrial pressure, pulse oximetry, and end-tidal CO<sub>2</sub>, *Pediatr. Crit. Care Med.*, 2011, **14**(5), S51–S61.
- 13 S. Hendrik, F. S. Magnet, M. Dreher and W. Windisch, Transcutaneous monitoring as a replacement for arterial PCO<sub>2</sub> monitoring during nocturnal non-invasive ventilation, *Respir. Med.*, 2011, **105**(1), 143–150.
- 14 J. E. Cotes, C. J. David and M. R. Miller, *Lung function: physiology, measurement and application in medicine*, John Wiley & Sons, Hoboken, USA, 2009.
- 15 R. Chen, A. D. Farmery, A. Obeid and C. E. W. Hahn, A Cylindrical-Core Fiber-Optic Oxygen Sensor Based on Fluorescence Quenching of a Platinum Complex Immobilized in a Polymer Matrix, *IEEE Sens. J.*, 2012, **12**(1), 71–75.
- 16 X. Yang, L. Peng, L. Yuan, P. Teng, F. Tian, L. Li and S. Luo, Oxygen gas optrode based on microstructured polymer optical fiber segment, *Opt. Commun.*, 2011, **284**, 3462–3466.
- 17 R. M. Bukowski, R. Ciriminna, M. Pagliaro and F. V. Bright, High-Performance Quenchometric Oxygen Sensors Based on Fluorinated Xerogels Doped with [Ru(dpp)<sub>3</sub>]<sup>2+</sup>, *Anal. Chem.*, 2005, **77**, 2670–2672.
- 18 P. K. Contreras-Gutierrez, S. Medina-Rodríguez, A. L. Medina-Castillo, J. F. Fernandez-Sanchez and A. Fernandez-Gutierrez, A new highly sensitive and versatile optical sensing film for controlling CO<sub>2</sub> in gaseous and aqueous media, *Sens. Actuators, B*, 2013, **184**, 281–287.
- 19 H. Segawa, E. Ohnishi, Y. Arai and K. Yoshida, Sensitivity of fiber-optic carbon dioxide sensors utilizing indicator dye, *Sens. Actuators, B*, 2003, **94**, 276–281.
- 20 J. F. Fernandez-Sanchez, R. Cannas, S. Spichiger, R. Steiger and U. E. Spichiger-Keller, Optical CO<sub>2</sub>-sensing layers for clinical application based on pH-sensitive indicators incorporated into nanoscopic metal-oxide supports, *Sens. Actuators, B*, 2007, **128**, 145–153.
- 21 O. Oter, K. Ertekin and S. Derinkuyu, Ratiometric sensing of CO<sub>2</sub> in ionic liquid modified ethyl cellulose matrix, *Talanta*, 2008, **76**, 557–563.
- 22 L. J. Crawford and N. R. Edmonds, Calculation of thickness for dip coated antireflective films, *Thin Solid Films*, 2006, **515**, 907–910.
- 23 M. M. Chen, X. M. Zhang, J. P. Ma, S. C. Chen, W. X. Chen and L. F. Feng, Experimental study on film thickness and the problem of free surface film flow in dip coating, *Asia-Pac. J. Chem. Eng.*, 2016, **11**, 695–704.
- 24 A. Thakur, P. Thakur and K. Yadav, Thickness Dependent Optical Properties of PEMA and (PEMA)<sub>0.85</sub>/(ZnO)<sub>0.15</sub> Nanocomposite Films Deposited by Spray Pyrolysis Technique on ITO Substrate, *AIP Conf. Proc.*, 2016, **1728**, 020412, DOI: 10.1063/1.4946463.